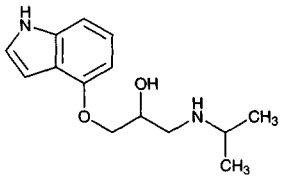
# Pindolol



**Molecular formula:** $C_{14}H_{20}N_2O_2$
**Molecular weight:** 248.33
**CAS Registry No.:** 13523-86-9
**Merck Index:** 7597
**Lednicer No.:** 2 342

## SAMPLE

**Matrix:** blood
**Sample preparation:** 1 mL Serum + 25 μL 5 μg/mL metoprolol in water + 100 μL 2 M NaOH + 4 mL dichloromethane, vortex for 10 s, centrifuge at 3000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 80 μL aliquot.

## HPLC VARIABLES

**Guard column:** NewGuard C18 (Brownlee)
**Column:** 250 × 4.6 5 μm Dynamax Microsorb C18
**Mobile phase:** MeCN:0.1% triethylamine in water adjusted to pH 3.5 with 85% phosphoric acid 20:80
**Flow rate:** 1
**Injection volume:** 80
**Detector:** F ex 215

## CHROMATOGRAM

**Retention time:** 7.04
**Internal standard:** metoprolol (11.65)
**Limit of detection:** 2 ng/mL
**Limit of quantitation:** 5 ng/mL

## KEY WORDS

serum

## REFERENCE

Chmielowiec,D.; Schuster,D.; Gengo,F. Determination of pindolol in human serum by HPLC, *J.Chromatogr.Sci.*, **1991**, *29*, 37–39.

## SAMPLE

**Matrix:** blood
**Sample preparation:** Condition a 1 mL 50 mg Bond Elut 40 μm cynaopropylsilica SPE cartridge with 1 mL MeOH at 6 mL/min and with 1 mL pH 7.4 buffer at 6 mL/min. Centrifuge plasma, add 1 mL plasma at 0.18 mL/min to the SPE cartridge, wash with 1 mL pH 7.4 buffer at 1.5 mL/min, elute with 300 μL MeOH:2-aminoheptane 99.9:0.1 at 1.5 mL/min, pass 700 μL pH 3.0 buffer through the cartridge at 1.5 mL/min. Mix both eluates, inject a 250 μL aliquot. (pH 7.4 Buffer was 250 mL 100 mM $KH_2PO_4$ and 195.5 mL 100 mM NaOH, made up to 1 L, if necessary pH adjusted to 7.4. pH 3.0 Buffer was 4 g NaOH in 700 mL water, pH adjusted to 3.0 with 85% phosphoric acid, made up to 1 L with water.)

## HPLC VARIABLES

**Guard column:** 4 × 4 5 μm LiChrospher 100 RP-18
**Column:** 250 × 4 4 μm Superspher 100 RP-18 (Merck)
**Mobile phase:** MeOH:buffer 30:70 containing 0.5% 2-aminoheptane (Buffer was 4 g NaOH in 700 mL water, pH adjusted to 3.0 with 85% phosphoric acid, made up to 1 L with water.)
**Column temperature:** 37
**Flow rate:** 1.2
**Injection volume:** 250
**Detector:** F ex 255 em 315

## CHROMATOGRAM

**Retention time:** 10

**KEY WORDS**
plasma; SPE

**REFERENCE**
Hubert,P.; Chiap,P.; Moors,M.; Bourguignon,B.; Massart,D.L.; Crommen,J. Knowledge-based system for the automated solid-phase extraction of basic drugs fom plasma coupled with their liquid chromatographic determination. Application to the biodetermination of β-receptor bocking agents, *J.Chromatogr.A*, **1994**, *665*, 87–99.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

**HPLC VARIABLES**
**Column:** 300 × 3.9 4 μm NovaPack C18
**Mobile phase:** MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) $KH_2PO_4$ adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
**Column temperature:** 30
**Flow rate:** 0.8
**Injection volume:** 50
**Detector:** UV 265

**CHROMATOGRAM**
**Retention time:** 3.98
**Limit of detection:** <120 ng/mL

**KEY WORDS**
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvox-

amine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

### REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

### SAMPLE

**Matrix:** blood, gastric contents, tissue, urine

**Sample preparation:** 1 mL Blood, urine, or gastric contents or 1 g tissue homogenate + 500 μL buffer + 8 mL n-hexane:ethyl acetate 70:30, mix on a rotary mixer for 10 min, centrifuge at 3000 g for 8 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL 12.5 mM NaOH in MeOH:water 50:50, inject a 50 μL aliquot. (Buffer was 13.8 g potassium carbonate in 100 mL water, pH adjusted to 9.5 with concentrated HCl.)

### HPLC VARIABLES

**Guard column:** 4 × 4 30 μm LiChrocart Aluspher RP-select B (Merck)

**Column:** 125 × 4 5 μm Aluspher RP-select B (Merck)

**Mobile phase:** Gradient. A was 12.5 mM NaOH in MeOH. B was 12.5 mM NaOH in water. A:B 10:90 for 5 min, to 90:10 over 15 min, maintain at 90:10 for 5 min, return to initial conditions over 1 min, re-equilibrate for 5 min.

**Flow rate:** 1

**Injection volume:** 50

**Detector:** UV 230, 254

### CHROMATOGRAM

**Retention time:** 9

### OTHER SUBSTANCES

**Extracted:** alprenolol, amitriptyline, bromazepam, carbamazepine, chlordiazepoxide, chlorpromazine, clonazepam, desipramine, diazepam, flunitrazepam, haloperidol, nitrendipine, nordiazepam, nortriptyline, zolpidem

**Also analyzed:** acebutolol, acetaminophen, alprazolam, amphetamine, atenolol, betaxolol, brotizolam, caffeine, camazepam, captopril, chloroquine, clobazam, clomipramine, clothiapine, clotiazepam, cloxazolam, cocaine, codeine, diclofenac, dihydralazine, dihydrocodeine, dihydroergotamine, diphenhydramine, domperidone, doxepin, droperidol, ergotamine, ethyl loflazepate, fenethylline, fluoxetine, flupentixol, flurazepam, furosemide, gliclazide, hydrochlorothiazide, hydroxyzine, ibuprofen, imipramine, ketazolam, loprazolam, lorazepam, lormetazepam, maprotiline, medazepam, mepyramine, methadone, methaqualone, methyldopa, methylphenidate, metoclopramide, metoprolol, mexiletine, mianserin, midazolam, minoxidil, morphine, nadolol, nitrazepam, oxprenolol, papaverine, pentazocine, phenprocoumon, phenylbutazone, pipamperone, piritramide, practolol, prazepam, prazosin, promazine, promethazine, propoxyphene, propranolol, prothipendyl, quinine, sotalol, sulpride, thioridazine, trazodone, triazolam, trimipramine, tripelennamine, tyramine, verapamil, yohimbine

### REFERENCE

Lambert,W.E.; Meyer,E.; De Leenheer,A.P. Systematic toxicological analysis of basic drugs by gradient elution of an alumina-based HPLC packing material under alkaline conditions, *J.Anal.Toxicol.*, **1995**, *19*, 73–78.

### SAMPLE

**Matrix:** blood, urine

**Sample preparation:** Dilute urine 1:10 with water. 500 μL Plasma or diluted urine + 500 μL buffer + 50 μL 2.2 μg/mL alprenolol in water, vortex, add 3 mL diethyl ether, shake for 10 min, centrifuge at 250 g for 4 min. Remove the ether layer and add it to 100 μL dilute sulfuric acid (pH 2.2), vortex for 1 min, centrifuge at 250 g for 4 min, inject a 20-75 μL aliquot of the aqueous phase. (Buffer was 5.3 g sodium bicarbonate and 4.2 g sodium carbonate in 100 mL water, pH 9.5.)

### HPLC VARIABLES

**Column:** 300 × 4 10 μm Micropak CN-10 alkylnitrile (Varian) (Prepare column by rinsing with 100 mL dichloromethane, with 100 mL MeCN:water 50:50, and with mobile phase.)
**Mobile phase:** 10 mM $KH_2PO_4$ adjusted to pH 2.6 with concentrated phosphoric acid
**Flow rate:** 2
**Injection volume:** 20-75
**Detector:** UV 220

### CHROMATOGRAM

**Retention time:** 5.5
**Internal standard:** alprenolol (9.5)
**Limit of detection:** 1.2 ng

### OTHER SUBSTANCES

**Simultaneous:** atenolol, caffeine, disopyramide, nadolol, oxprenolol, practolol, procainamide, pronethalol, propranolol, sotalol, timolol
**Interfering:** N-acetylprocainamide, lidocaine, quinidine

### KEY WORDS

plasma

### REFERENCE

Shields,B.J.; Lima,J.J.; Binkley,P.F.; Leier,C.V.; MacKichan,J.J. Determination of pindolol in human plasma and urine by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1986**, *378*, 163–171.

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### SAMPLE

**Matrix:** blood, urine
**Sample preparation:** Serum. Condition a 1 mL diol (2OH) SPE cartridge (Varian) with two volumes of MeOH and two volumes of water. Add 1 mL serum to the SPE cartridge, wash with 250 μL water, elute with two 500 μL aliquots of MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 250 μL mobile phase, inject a 100 μL aliquot. Urine. Centrifuge urine at 2000 g for 10 min, inject a 20 μL aliquot of the supernatant.

### HPLC VARIABLES

**Column:** 250 × 4.6 10 μm Chiralcel OD-R
**Mobile phase:** MeCN:300 mM sodium perchlorate 40:60
**Flow rate:** 0.5
**Injection volume:** 100
**Detector:** F ex 270 em 310

### CHROMATOGRAM

**Retention time:** 10.5 (R-(+)), 18.5 (S-(-))
**Limit of detection:** 76 ng/mL (S, urine), 21 ng/mL (R, urine), 4.3 ng/mL (S, serum), 1.2 ng/mL (R, serum)

### KEY WORDS

serum; chiral; SPE

### REFERENCE

Zhang,H.; Stewart,J.T.; Ujhelyi,M. High-performance liquid chromatographic analysis of pindolol enantiomers in human serum and urine using a reversed-phase cellulose-based chiral column, *J.Chromatogr.B*, **1995**, *668*, 309–313.

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### SAMPLE

**Matrix:** blood, urine
**Sample preparation:** Filter (0.45 μm) plasma or urine. Inject a 20 (plasma) or 50 (urine) μL aliquot on to column A and elute to waste, after 5 min backflush the contents of column A on to column B with mobile phase B, elute with mobile phase B, monitor the effluent from column B. Re-equilibrate column A with mobile phase A for 5 min before the next run.

**HPLC VARIABLES**
**Column:** A 10 × 4.6 internal-surface phenylboronic acid (Yokogawa Analytical Systems); B 150 × 4.6 Capcell Pak C18 SG120 (Shiseido)
**Mobile phase:** A MeOH:50 mM $Na_2HPO_4$ 5:95; B MeOH:50 mM pH 2.0 phosphate buffer 20:80
**Flow rate:** 1
**Injection volume:** 20-50
**Detector:** F ex 255 em 315 or UV 255

**CHROMATOGRAM**
**Retention time:** 6
**Limit of detection:** 100 nM

**OTHER SUBSTANCES**
**Noninterfering:** acetaminophen, caffeine, furosemide, hydrochlorothiazide, nalidixic acid, norfloxacin, pipemidic acid, phenylbutazone, salicylic acid, theophylline, tolbutamide, warfarin

**KEY WORDS**
plasma; column-switching

**REFERENCE**
Ohta,T.; Niida,S.; Nakamura,H. Selective extraction of β-blockers from biological fluids by column-switching high-performance liquid chromatography using an internal-surface phenylboronic acid precolumn, *J.Chromatogr.B*, **1996**, *675*, 168–173.

**SAMPLE**
**Matrix:** blood, urine
**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

**HPLC VARIABLES**
**Guard column:** 20 mm long Symmetry C18
**Column:** 250 × 4.6 5 μm Symmetry C8 (Waters)
**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
**Column temperature:** 30
**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
**Injection volume:** 10-30
**Detector:** UV 215.8

**CHROMATOGRAM**
**Retention time:** 8.568

**KEY WORDS**
whole blood

**REFERENCE**
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

**SAMPLE**
**Matrix:** bulk

**Sample preparation:** Dissolve 1.5 mg compound in 1 mL reagent, add 3 μL triethylamine, sonicate for 20 min, add 3 μL diethylamine, let stand for 15 min, inject an aliquot. (Reagent was 2 mg/mL (R)-(-)-(naphth-1-yl)ethylisocyanate solution in dry chloroform:DMF 80:20.)

**HPLC VARIABLES**
**Column:** 200 × 4.6 Silica 100 RP 18
**Mobile phase:** MeOH:water 70:30
**Flow rate:** 1.5
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** k' 2.50, k' 2.87 (enantiomers)

**OTHER SUBSTANCES**
**Also analyzed:** atenolol, methylphenidate, metipranolol, propranolol, propylhexedrine, talinolol

**KEY WORDS**
derivatization

**REFERENCE**
Jira,T.; Toll,C.; Vogt,C.; Beyrich,T. Zur Trennung einiger racemischer β-Blocker und α-Sympathikomimetika durch HPLC nach Derivatisierung [The separation of some racemic β-blockers and α-sympathomimetics with HPLC following derivatization], *Pharmazie*, **1991**, *46*, 432–434.

**SAMPLE**
**Matrix:** bulk
**Sample preparation:** Dissolve 5 mg amino acids in 10 mL MeCN:water:triethylamine 50:50:0.55. Remove a 50 μL aliquot and add it to 50 μL 0.66% 2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl isothiocyanate (Fluka) in MeCN, shake mechanically for 30 min, add 10 μL 0.26% ethanolamine in MeCN, shake for 10 min, make up to 1 mL with MeCN, inject a 10 μL aliquot.

**HPLC VARIABLES**
**Column:** 25 × 4 (sic) 5 μm LiChrospher 100 RP-18
**Mobile phase:** MeOH:water 85:15
**Flow rate:** 0.5
**Injection volume:** 10
**Detector:** UV 231

**CHROMATOGRAM**
**Retention time:** k' 3.00, k' 3.71 (enantiomers)

**KEY WORDS**
derivatization; chiral

**REFERENCE**
Lobell,M.; Schneider,M.P. 2,3,4,6-Tetra-O-benzoyl-β-D-glucopyranosyl isothiocyanate: an efficient reagent for the determination of enantiomeric purities of amino acids, β-adrenergic blockers and alkyloxiranes by high-performance liquid chromatography using standard reversed-phase columns, *J.Chromatogr.*, **1993**, *633*, 287–294.

**SAMPLE**
**Matrix:** bulk
**Sample preparation:** Dissolve 10 μmole compound (as free base or hydrochloride) in 500 μL MeCN, add 250 μL 5% sodium carbonate (for hydrochlorides only), add 500 μL 100 mM reagent in MeCN, vortex for 1 min, heat at 60° for 2 h, add 100 μmole L-proline, heat at 60° for 30 min. Remove a 100 μL aliquot and dilute it with mobile phase, neutralize with acetic acid, inject a 10 μL aliquot. Prepare the reagent ((R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexyl-isothiocyanate) as follows. Add 0.7 mL carbon disulfide to 6 mL (1R,2R)-(-)-1,2-diaminocyclohexane, 12 mL water, and 12 mL EtOH, heat the oil bath to 80°, add 2.8 mL carbon disulfide dropwise (making sure that the product does not start to precipitate), when addition is complete reflux for 1 h, acidify with 500 μL 5 M HCl, reflux for 12 h, cool, filter, wash the solid with a

little cold EtOH to give trans-4,5-tetramethyleneimidazolidine-2-thione as a white fluffy solid (mp 148-150°) (Tetrahedron 1993, 49, 4419). Stir 7.97 g 3,5-dinitrobenzoyl chloride in 30 mL dichloroethane at 50°, add a solution of 6 g trans-4,5-tetramethyleneimidazolidine-2-thione in 120 mL dichloroethane containing a catalytic amount of 4-(dimethylamino)pyridine over 15 min, reflux for 2 h, remove the crystals of (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate by filtration, evaporate the filtrate to dryness and dissolve the residue in 60 mL dichloroethane, reflux for 16 h to obtain more (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate (mp >250°, $[\alpha]_{546}$ = -133° (c = 1) in MeCN).

### HPLC VARIABLES
**Column:** 125 × 4 5 μm Lichrospher 60 RP Select B
**Mobile phase:** MeCN:20 mM ammonium acetate 55:45
**Flow rate:** 1
**Injection volume:** 10
**Detector:** UV 254

### CHROMATOGRAM
**Retention time:** k' 3.91, k' 5.59 (enantiomers)

### OTHER SUBSTANCES
**Also analyzed:** acebutolol, alprenolol, atenolol, carazolol, carvedilol, formoterol, methamphetamine, metipranolol, metoprolol, nifenanol, nitrilo atenolol, oxprenolol, propranolol, xamoterol

### KEY WORDS
derivatization; chiral

### REFERENCE
Kleidernigg,O.P.; Posch,K.; Lindner,W. Synthesis and application of a new isothiocyanate as a chiral derivatizing agent for the indirect resolution of chiral amino alcohols and amines, *J.Chromatogr.A*, **1996**, *729*, 33–42.

## SAMPLE
**Matrix:** formulations
**Sample preparation:** Take up in mobile phase, inject an aliquot.

### HPLC VARIABLES
**Column:** 250 × 4.6 10 μm LiChrosorb C2
**Mobile phase:** MeCN:buffer 35:65 (1 mL 100 mM HCl + 1200 mL water + 5.84 g NaCl, mix to dissolve, add 700 mL MeOH, make up to 2 L, apparent pH 4.5.)
**Flow rate:** 1.2
**Injection volume:** 20
**Detector:** UV 254

### CHROMATOGRAM
**Retention time:** 7.8

### OTHER SUBSTANCES
**Simultaneous:** atenolol, nadolol, alprenolol, acebutolol, oxprenolol, metoprolol, practolol, propranolol, timolol
**Interfering:** sotalol

### KEY WORDS
tablets

### REFERENCE
Patel,B.R.; Kirschbaum,J.J.; Poet,R.B. High-pressure liquid chromatography of nadolol and other β-adrenergic blocking drugs, *J.Pharm.Sci.*, **1981**, *70*, 336–338.

## SAMPLE
**Matrix:** saliva

**Sample preparation:** Condition a 100 mg 1 mL Bond-Elut C2 SPE cartridge with 1 mL MeOH, 1 mL water, and 1 mL pH 9.0 borate buffer. Centrifuge a cotton roll soaked with saliva at 1000 g for 5 min, remove the liquid supernatant. 1 mL Supernatant + 50 μL 100 μg/mL tertatolol, add to the SPE cartridge, wash with 500 μL water, wash with 500 μL MeCN, elute with two 500 μL portions of acidified MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 50 μL mobile phase, mix for 15 s, inject a 40 μL aliquot. (Acidified MeOH was 50 mL MeOH + 300 μL 96% acetic acid.)

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**HPLC VARIABLES**
**Guard column:** RCSS silica guard-pack (Waters)
**Column:** 250 × 4.6 Chiralcel OD-H
**Mobile phase:** n-Hexane:EtOH:diethylamine 50:50:1
**Flow rate:** 1
**Injection volume:** 40
**Detector:** F ex 225 em 290 cut-off filter

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**CHROMATOGRAM**
**Internal standard:** (R,S)-tertatolol

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**KEY WORDS**
SPE; chiral

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**REFERENCE**
Höld,K.M.; de Boer,D.; Zuidema,J.; Maes,R.A.A. Evaluation of the Salivette as sampling device for monitoring β-adrenoceptor blocking drugs in saliva, *J.Chromatogr.B*, **1995**, *663*, 103–110.

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**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Dissolve in MeOH, dilute with mobile phase.

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**HPLC VARIABLES**
**Column:** 150 × 3.9 Novapak-phenyl-4
**Mobile phase:** MeOH:15 mM pH 6.5 sodium acetate buffer 81:19
**Flow rate:** 1.0
**Injection volume:** 10
**Detector:** UV 254

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**CHROMATOGRAM**
**Retention time:** 2.5

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**OTHER SUBSTANCES**
**Simultaneous:** perphenazine

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**REFERENCE**
Al-Obaid,A.M.; Hagga,M.E.M.; El-Khawad,I.E.; El-Mahi,O.H.M. Simultaneous quantitation of some phenothiazine drug substances and their monosulphoxide degrades by high performance liquid chromatography (HPLC), *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1369–1389.

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**SAMPLE**
**Matrix:** solutions

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**HPLC VARIABLES**
**Column:** 150 × 4.6 bidentate C18 silane column (Preparation is as follows. Reflux 60 g 7 μm Zorbax PSM300 silica in 600 mL 75 ppm HF in water for 72 h (Caution! HF is highly toxic!), wash with 1.5 L water, wash with 500 mL acetone, dry overnight under vacuum (30 in. Hg). Add to 570 mL water boil for 10 h, cool to room temperature, wash with 500 mL acetone, dry overnight at 110° under vacuum (30 in. Hg). Heat 6 g of this material at 110° under vacuum (30 in. Hg) and place it in a dry nitrogen atmosphere. Add 60 mL dry xylene, 240 μL pyridine, and 4.9 mL dichlorodimethyldioctadecyldisiloxane (?) (Petrarch Systems, Bristol, PA). Reflux under nitrogen for 80 h, cool, wash with 300 mL toluene, 300 mL dichloromethane, 300 mL MeOH, 300 mL MeOH:water 50:50, and 300 mL acetone. Dry at 110° under vacuum (30 in. Hg overnight) (cf. US Pat. 4 746 572).)

**Mobile phase:** MeCN:17 mM pH 11 $K_3PO_4$ buffer 50:50
**Column temperature:** 40
**Flow rate:** 1
**Injection volume:** 5
**Detector:** not given

### CHROMATOGRAM
**Retention time:** 1.9

### OTHER SUBSTANCES
**Simultaneous:** metoprolol

### REFERENCE
Kirkland,J.J.; van Straten,M.A.; Claessens,H.A. Reversed-phase high-performance liquid chromatography of basic compounds at pH 11 with silica-based column packings, *J.Chromatogr.A*, **1998**, *797*, 111–120.

### SAMPLE
**Matrix:** solutions
**Sample preparation:** Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

### HPLC VARIABLES
**Column:** 125 × 4.9 Spherisorb S5W silica
**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
**Flow rate:** 2
**Injection volume:** 20
**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

### CHROMATOGRAM
**Retention time:** 1.8

### OTHER SUBSTANCES
**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, qui-

nine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

### REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

### SAMPLE

**Matrix:** solutions

### HPLC VARIABLES

**Column:** 250 × 4.6 cellulose tris(3,5-dimethylphenylcarbamate)
**Mobile phase:** Hexane:isopropanol:diethylamine 80:20:0.1
**Flow rate:** 0.5
**Detector:** UV

### CHROMATOGRAM

**Retention time:** k' 3.17 (of first (+) enantiomer)

### KEY WORDS

chiral; α 5.07

### REFERENCE

Okamoto,Y.; Aburatani,R.; Hatano,K.; Hatada,K. Optical resolution of racemic drugs by chiral HPLC on cellulose and amylose tris(phenylcarbamate) derivatives, *J.Liq.Chromatogr.*, **1988**, *11*, 2147–2163.

### SAMPLE

**Matrix:** solutions
**Sample preparation:** Filter (0.22 μm), inject a 10 μL aliquot.

### HPLC VARIABLES

**Column:** 250 × 4.6 internal surface reversed-phase silica (Pinkerton) (Regis Chemical)
**Mobile phase:** Isopropanol:100 mM pH 6.8 $KH_2PO_4$ 10:90
**Flow rate:** 1
**Injection volume:** 10
**Detector:** UV 232-274 (wavelength of maximum absorption used)

### CHROMATOGRAM

**Retention time:** 57.2

### OTHER SUBSTANCES

**Simultaneous:** carteolol, atenolol, metoprolol, oxprenolol, acebutolol, alprenolol

### REFERENCE

Ohshima,T.; Takagi,K.; Miyamoto,K.-I. High performance liquid chromatographic retention time of β-blockers as an index of pharmacological activity, *J.Liq.Chromatogr.*, **1993**, *16*, 3933–3939.

### SAMPLE

**Matrix:** solutions
**Sample preparation:** 50 μL Solution + 50 μL pH 7.4 PBS + 100 μL MeOH, centrifuge at 12000 g for 10 min, inject a 50 μL aliquot.

### HPLC VARIABLES

**Column:** 150 × 4.6 Cosmosil 5C18-P (Nacalai Tesque)
**Mobile phase:** MeOH:50 mM $NaH_2PO_4$ 25:75
**Flow rate:** 1

**Injection volume:** 50
**Detector:** UV 250

**CHROMATOGRAM**
**Internal standard:** pindolol

**OTHER SUBSTANCES**
**Simultaneous:** carteolol

**KEY WORDS**
buffer; Earle's balanced salt solution; pindolol is IS

**REFERENCE**
Sasaki,H.; Igarishi,Y.; Nishida,K.; Nakamura,J. Intestinal permeability of ophthalmic β-blockers for predicting ocular permeability, *J.Pharm.Sci.*, **1994**, *83*, 1335–1338.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Inject a 40 μL aliquot.

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm Hypersil MOS C-8
**Mobile phase:** MeOH:water 70:30 containing 0.02% dimethyloctylamine, 25 mM sodium hexanesulfonate, and 20 mM acetic acid
**Flow rate:** 1
**Injection volume:** 40
**Detector:** F ex 275 em 305

**CHROMATOGRAM**
**Retention time:** 4.6

**OTHER SUBSTANCES**
**Simultaneous:** alprenolol, atenolol, propranolol (UV 288)

**REFERENCE**
Adson,A.; Burton,P.S.; Raub,T.J.; Barsuhn,C.L.; Audus,K.L.; Ho,N.F.H. Passive diffusion of weak organic electrolytes across Caco-2 cell monolayers: Uncoupling the contributions of hydrodynamic, transcellular, and paracellular barriers, *J.Pharm.Sci.*, **1995**, *84*, 1197–1204.

**SAMPLE**
**Matrix:** solutions

**HPLC VARIABLES**
**Column:** 62 × 2 packed with chiral packing (Prepare packing by dissolving 3-chloro-4-methylphenylcarbamate cellulose in THF, coat on Nucleosil 1000-7, dry at 60° for 3 h under reduced pressure.)
**Mobile phase:** Hexane:isopropanol 90:10
**Flow rate:** 0.1
**Injection volume:** 20
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** k' 9.50

**KEY WORDS**
narrow-bore; chiral; α 2.14

**REFERENCE**
Chankvetadze,B.; Chankvetadze,L.; Sidamonidze,S.; Yashima,E.; Okamoto,Y. Enantioseparation of some chiral pharmaceuticals using narrow-bore liquid chromatography, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 695–699.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Inject a 20 μL aliquot of a 1 mg/mL solution.

**HPLC VARIABLES**
**Column:** 250 × 4.6 10 μm Chiralcel OD
**Mobile phase:** Hexane:isopropanol:diethylamine 20:80:0.1
**Flow rate:** 0.5
**Injection volume:** 20
**Detector:** UV 265

**CHROMATOGRAM**
**Retention time:** k' 0.25, 1.66 (enantiomers)

**KEY WORDS**
chiral

**REFERENCE**
Ekelund,J.; van Arkens,A.; Bronnum-Hansen,K.; Fich,K.; Olsen,L.; Petersen,P.V. Chiral separations of β-blocking drug substances using chiral stationary phases, *J.Chromatogr.A*, **1995**, *708*, 253–261.

**SAMPLE**
**Matrix:** solutions

**HPLC VARIABLES**
**Column:** 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
**Mobile phase:** MeCN:100 mM pH 7.0 phosphate buffer 20:80
**Flow rate:** 1
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** k' 3.85

**OTHER SUBSTANCES**
**Also analyzed:** acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

**REFERENCE**
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on $\alpha_1$-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

**SAMPLE**
**Matrix:** solutions

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
**Mobile phase:** MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
**Flow rate:** 0.6
**Injection volume:** 25
**Detector:** UV 229

**CHROMATOGRAM**
**Retention time:** 7.02 (A), 4.07 (B)

## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

## KEY WORDS

details of plasma extraction

## REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

## SAMPLE

**Matrix:** solutions

## HPLC VARIABLES

**Column:** 150 × 4.6 cellulose 3,5-dimethylphenylcarbamate/10-undecenoate bonded to allylsilica
**Mobile phase:** Heptane:isopropanol:diethylamine 80:20:0.1
**Flow rate:** 1
**Injection volume:** 1000
**Detector:** UV 254

## CHROMATOGRAM

**Retention time:** k' 5.51

## KEY WORDS

chiral; α 2.84

## REFERENCE

Oliveros,L.; Lopez,P.; Minguillon,C.; Franco,P. Chiral chromatographic discrimination ability of a cellulose 3,5-dimethylphenylcarbamate/10-undecenoate mixed derivative fixed on several chromatographic matrices, *J.Liq.Chromatogr.*, **1995**, *18*, 1521–1532.

## SAMPLE

**Matrix:** solutions

**Sample preparation:** Prepare a 1-10 μg/mL solution in water, inject an aliquot.

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm Hypersil SCX/C18
**Mobile phase:** MeCN:25 mM pH 3 $Na_2HPO_4$ 50:50
**Injection volume:** 20
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** k' 2.35

**OTHER SUBSTANCES**
**Also analyzed:** amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenol, phenolphthalein, propranolol, resorcinol, salicylic acid, secobarbital, terbutaline, xylazine

**KEY WORDS**
effect of mobile phase pH on capacity factor is discussed

**REFERENCE**
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

**SAMPLE**
**Matrix:** solutions

**HPLC VARIABLES**
**Guard column:** 10 × 3.2 5 μm Partisil ODS3
**Column:** 100 × 4.6 5 μm Partisil ODS3
**Mobile phase:** MeCN:buffer 15:85 (Buffer was 60 mM $KH_2PO_4$ adjusted to pH 3.0 with phosphoric acid.)
**Flow rate:** 0.6-1
**Injection volume:** 10-100
**Detector:** UV 270

**REFERENCE**
Palm,K.; Luthman,K.; Ungell,A.-L.; Strandlund,G.; Artursson,P. Correlation of drug absorption with molecular surface properties, *J.Pharm.Sci.*, **1996**, *85*, 32–39.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Dissolve salt of pindolol in MeOH:water 50:50, inject an aliquot.

**HPLC VARIABLES**
**Column:** 200 × 4.6 5 μm Hypersil RP-18
**Mobile phase:** Gradient. MeOH:buffer from 20:80 to 80:20 over 10 min. (Buffer was 2% acetic acid containing 1.1% sodium 1-heptanesulfonate.)
**Column temperature:** 40
**Flow rate:** 1.5
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** 7.8

**OTHER SUBSTANCES**
**Simultaneous:** benzoic acid (UV 273), 2-methoxyphenylacetic acid (UV 270)

**REFERENCE**
Pietiläinen,H.; Saesmaa,T. HPLC determination of pindolol benzoate and pindolol 2-methoxyphenylacetate, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 583–591.

## SAMPLE

**Matrix:** solutions

**Sample preparation:** Mix a 100 μL of a 10 μM solution in MeCN:water:triethylamine 50:50:0.1 with 100 μL 1 mM (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in MeCN, heat in the dark at 65° for 1.5 h, inject an aliquot. (Synthesis of (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene from EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate: benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ TLC plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane: acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-aminopyrrolidine is also reported to be available from Tokyo Kasei. Add 100 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-10°, stir at room temperature for 30 min, remove the MeCN by evaporation under reduced pressure, dissolve the residue in 50 mL 5% HCl, wash 3 times with 50 mL portions of ethyl acetate, adjust the pH of the aqueous solution to 13-14 with 5% NaOH, extract 6 times with 50 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as orange crystals (mp 96-98°) (Analyst 1992, 117, 727). Add 100 μL thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25

mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as yellow crystals (mp 160-170° d) (Analyst 1995, 120, 385).)

**HPLC VARIABLES**
**Column:** 150 × 4.6 5 μm Inertsil ODS-80A
**Mobile phase:** MeCN:water:trifluoroacetic acid 44:56:0.1
**Column temperature:** 40
**Flow rate:** 1
**Detector:** F ex 460 em 550

**CHROMATOGRAM**
**Retention time:** 30.9, 39.4 (enantiomers)
**Limit of detection:** 296-320 fmole

**KEY WORDS**
derivatization; chiral

**REFERENCE**
Toyo'oka,T.; Toriumi,M.; Ishii,Y. Enantioseparation of β-blockers labelled with a chiral fluorescent reagent, R(-)-DBD-PyNCS, by reversed-phase liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1467–1476.

**SAMPLE**
**Matrix:** urine
**Sample preparation:** 1 mL Urine + 10 mg β-glucuronidase/arylsulfatase (Helix pomatia, Sigma), heat at 37° overnight, add an equal volume of buffer, centrifuge at 2000 g for 5 min, inject an aliquot of the supernatant onto column A with mobile phase A and elute to waste. After 2.5 min backflush the contents of column A onto column B with mobile phase B, monitor the effluent from column B. Re-equilibrate both columns for 12.5 min before the next injection. (Buffer was 200 mM boric acid adjusted to pH 9.5 with 5 M NaOH.)

**HPLC VARIABLES**
**Column:** A 10 × 4.6 5 μm Spherisorb cyanopropyl; B 250 × 4.6 Capcell Pak C18 UG-120 (Shiseido)
**Mobile phase:** A water; B Gradient. MeCN:buffer from 3:97 to 30:70 over 30 min, to 40:60 over 8 min (Buffer was 3.4 mL/L phosphoric acid adjusted to pH 3.0 with 5 M NaOH.)
**Flow rate:** A 1.25; B 1
**Injection volume:** 100
**Detector:** UV 220

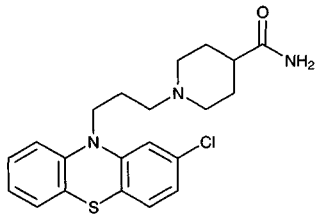
**CHROMATOGRAM**
**Retention time:** 10.3
**Limit of detection:** 250 ng/mL

**OTHER SUBSTANCES**
**Extracted:** acebutolol, alprenolol, amphetamine, atenolol, bopindolol, codeine, ephedrine, labetalol, metoprolol, morphine, nadolol, oxprenolol, propranolol, timolol

**KEY WORDS**
column-switching

**REFERENCE**
Saarinen,M.T.; Sirén,H.; Riekkola,M.-L. Screening and determination of β-blockers, narcotic analgesics and stimulants in urine by high-performance liquid chromatography with column switching, *J.Chromatogr.B*, **1995**, *664*, 341–346.

# Pipamazine



**Molecular formula:** $C_{21}H_{24}ClN_3OS$
**Molecular weight:** 401.96
**CAS Registry No.:** 84-04-8
**Merck Index:** 7607
**Lednicer No.:** 1 385

## SAMPLE

**Matrix:** solutions
**Sample preparation:** Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

## HPLC VARIABLES

**Column:** 125 × 4.9 Spherisorb S5W silica
**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
**Flow rate:** 2
**Injection volume:** 20
**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

## CHROMATOGRAM

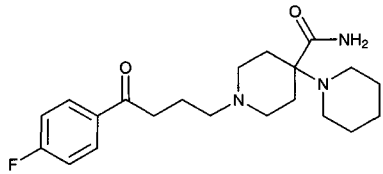
**Retention time:** 2.25

## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

**REFERENCE**
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

# Pipamperone



**Molecular formula:** $C_{21}H_{30}FN_3O_2$
**Molecular weight:** 375.49
**CAS Registry No.:** 1893-33-0, 2448-68-2 (2.HCl)
**Merck Index:** 7608
**Lednicer No.:** 2 288

## SAMPLE

**Matrix:** blood
**Sample preparation:** 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

## HPLC VARIABLES

**Column:** 300 × 3.9 4 μm NovaPack C18
**Mobile phase:** MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) $KH_2PO_4$ adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
**Column temperature:** 30
**Flow rate:** 0.8
**Injection volume:** 50
**Detector:** UV 246

## CHROMATOGRAM

**Retention time:** 4.98
**Limit of detection:** <120 ng/mL

## KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine;

diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

## REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

## SAMPLE

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

## HPLC VARIABLES

**Guard column:** 20 mm long Symmetry C18

**Column:** 250 × 4.6 5 μm Symmetry C8 (Waters)

**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

**Column temperature:** 30

**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

**Injection volume:** 10-30

**Detector:** UV 200.5

## CHROMATOGRAM

**Retention time:** 10.918

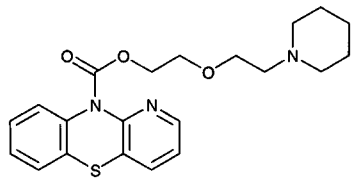
## KEY WORDS

whole blood

## REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

# Pipazethate



**Molecular formula:** $C_{21}H_{25}N_3O_3S$
**Molecular weight:** 399.51
**CAS Registry No.:** 2167-85-3, 6056-11-7 (HCl)
**Merck Index:** 7609
**Lednicer No.:** 1 390

## SAMPLE

**Matrix:** solutions
**Sample preparation:** Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

## HPLC VARIABLES

**Column:** 125 × 4.9 Spherisorb S5W silica
**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
**Flow rate:** 2
**Injection volume:** 20
**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

## CHROMATOGRAM

**Retention time:** 5.9

## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

**REFERENCE**
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

# Pipecuronium bromide

**Molecular formula:** $C_{35}H_{62}Br_2N_4O_4$
**Molecular weight:** 762.71
**CAS Registry No.:** 52212-02-9, 68399-57-5 (dihydrate)
**Merck Index:** 7612
**Lednicer No.:** 4 70

**SAMPLE**
**Matrix:** bulk

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm LiChrosorb Si 60
**Mobile phase:** MeCN:MeOH:concentrated ammonia solution 43:43:14 containing 100 mM ammonium carbonate and 100 mM ammonium chloride
**Flow rate:** 1
**Detector:** UV 213

**CHROMATOGRAM**
**Retention time:** 9

**OTHER SUBSTANCES**
**Simultaneous:** impurities

**KEY WORDS**
stability-indicating

**REFERENCE**
Szepesi,G.; Gazdag,M.; Mihályfi,K. Selection of high-performance liquid chromatographic methods in pharmaceutical analysis. III. Method validation, *J.Chromatogr.*, **1989**, *464*, 265–278.

**SAMPLE**
**Matrix:** bulk
**Sample preparation:** Prepare a 0.5% solution in the mobile phase, inject a 20 μL aliquot.

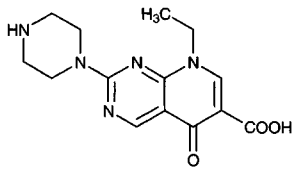
**HPLC VARIABLES**
**Column:** 250 × 4 5 μm SI 100 (Bio Separation Technologies)
**Mobile phase:** MeCN:100 mM sodium perchlorate 96:4
**Flow rate:** 1
**Injection volume:** 20
**Detector:** UV 213

**CHROMATOGRAM**
**Retention time:** 7
**Limit of detection:** 10 ng

**OTHER SUBSTANCES**
**Simultaneous:** impurities, vecuronium, pancuronium

**REFERENCE**
Gazdag,M.; Babják,M.; Kemenes-Bakos,P.; Görög,S. Analysis of steroids. XLI. Ion-pair high-performance liquid chromatographic separation of quaternary ammonium steroids on silica, *J.Chromatogr.*, **1991**, *550*, 639–644.

# Pipemidic acid




**Molecular formula:** $C_{14}H_{17}N_5O_3$
**Molecular weight:** 303.32
**CAS Registry No.:** 51940-44-4, 72571-82-5 (trihydrate)
**Merck Index:** 7613

**SAMPLE**
**Matrix:** blood
**Sample preparation:** Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate.

**HPLC VARIABLES**
**Column:** 250 × 4.6 Spherisorb ODS-2 endcapped
**Mobile phase:** MeCN:buffer 13:87 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)
**Column temperature:** 37
**Flow rate:** 1
**Detector:** UV 275

**CHROMATOGRAM**
**Retention time:** 4.57
**Internal standard:** ofloxacin (8.51)

**KEY WORDS**
plasma; ultrafiltrate

**REFERENCE**
Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

**SAMPLE**
**Matrix:** blood, tissue
**Sample preparation:** Plasma. Mix 100 μL plasma with 900 μL 100 mM phosphate buffer and 5 mL chloroform:ethyl chlorocarbonate 99:1, shake for 10 min, centrifuge at 1620 g for 5 min, evaporate the organic phase under reduced pressure, dissolve the residue in 100 μL MeOH:50 mM NaOH 2:1, inject a 20 μL aliquot. Tissue. Homogenate the cerebrum sample with 4 volumes of 100 mM phosphate buffer. Mix 1 mL homogenate with 5 mL dichloromethane, shake for 10 min, centrifuge at 1620 g for 5 min. Mix 4 mL 1 mM NaOH with 4 mL organic phase, shake for 10 min, centrifuge it at 1620 g for 5 min, collect 3 mL aqueous phase and treat in a manner similar to that for the plasma samples, except for the IS addition. Inject a 20 μL aliquot. (Caution! Chloroform is a carcinogen!)

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm Nucleosil 5 C 18

**Mobile phase:** MeOH:5 mM sodium dodecylsulfate adjusted to pH 2.5 with phosphoric acid
**Flow rate:** 0.8
**Injection volume:** 20
**Detector:** UV 280

**CHROMATOGRAM**
**Internal standard:** pipemidic acid

**OTHER SUBSTANCES**
**Extracted:** ciprofloxacin, foscarnet

**KEY WORDS**
plasma; brain; mouse; pipedimic acid is IS; derivatization

**REFERENCE**
Matsuo,H.; Ryu,M.; Nagata,A.; Uchida,T.; Kawakami,J.-I.; Yamamoto,K.; Iga,T.; Sawada,Y. Neurotoxicodynamics of the interaction between ciprofloxacin and foscarnet in mice, *Antimicrob.Agents Chemother.*, **1998**, *42*, 691–694.

**SAMPLE**
**Matrix:** solutions

**HPLC VARIABLES**
**Column:** Spherisorb ODS II
**Mobile phase:** MeCN containing 2 mM tetrabutyl ammonium hydrogen sulfate:100 mM citric acid buffer containing 5 mM ammonium perchlorate 87:13, adjusted to pH 2.2
**Flow rate:** 1.2
**Injection volume:** 5-50
**Detector:** F ex 290 em 460

**CHROMATOGRAM**
**Retention time:** 3.2

**OTHER SUBSTANCES**
**Simultaneous:** fleroxacin

**REFERENCE**
Uehlinger,G.E.; Schaedeli,F.; Kinzig,M.; Sörgel,F.; Frey,F.J. Pharmacokinetics of fleroxacin after multiple oral dosing in patients receiving regular hemodialysis, *Antimicrob.Agents Chemother.*, **1996**, *40*, 1903–1909.

**SAMPLE**
**Matrix:** urine
**Sample preparation:** Make up 1 mL urine to 25 mL with mobile phase, filter (0.45 μm). Inject a 20 μL aliquot.

**HPLC VARIABLES**
**Column:** 150 × 3.9 Nova-Pak C18
**Mobile phase:** MeCN:0.4 mM oxalic acid in water 28:72
**Flow rate:** 2.0
**Injection volume:** 20
**Detector:** F ex 270 em 440

**CHROMATOGRAM**
**Retention time:** 2.97
**Limit of detection:** 3.26 ng/mL

**OTHER SUBSTANCES**
**Extracted:** cinoxacin, oxolinic acid

**REFERENCE**
Durán Mer,I.; Galeano Díaz,T.; Rodríguez Cáceres,M.I.; Salinas López,F. Determination of the chemotherapeutic quinolonic and cinolonic derivatives in urine by high-performance liquid chromatography with ultraviolet and fluorescence detection in series, *J.Chromatogr.A*, **1997**, *787*, 119–127.

**SAMPLE**
**Matrix:** urine
**Sample preparation:** Make up 1 mL urine to 25 mL with mobile phase, filter (0.45 μm). Inject a 20 μL aliquot.

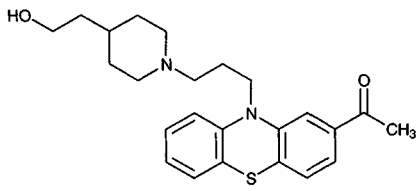
**HPLC VARIABLES**
**Column:** 150 × 3.9 Nova-Pak C18
**Mobile phase:** MeCN:400 μM oxalic acid in water 28:72
**Flow rate:** 2.0
**Injection volume:** 20
**Detector:** UV 265

**CHROMATOGRAM**
**Retention time:** 2.97
**Limit of detection:** 1.15 μg/mL

**OTHER SUBSTANCES**
**Simultaneous:** cinoxacin, nalidixic acid, oxolinic acid, piromidic acid

**REFERENCE**
Durán Meré,I.; Galeano Díaz,T.; Rodríguez Cáceres,M.I.; Salinas López,F. Determination of the chemotherapeutic quinolonic and cinolonic derivatives in urine by high-performance liquid chromatography with ultraviolet and fluorescence detection in series, *J.Chromatogr.A*, **1997**, *787*, 119–127.

# Piperacetazine




**Molecular formula:** $C_{24}H_{30}N_2O_2S$
**Molecular weight:** 410.58
**CAS Registry No.:** 3819-00-9
**Merck Index:** 7615
**Lednicer No.:** 1 386

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

**HPLC VARIABLES**
**Column:** 125 × 4.9 Spherisorb S5W silica
**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
**Flow rate:** 2
**Injection volume:** 20
**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

**CHROMATOGRAM**
**Retention time:** 2.5

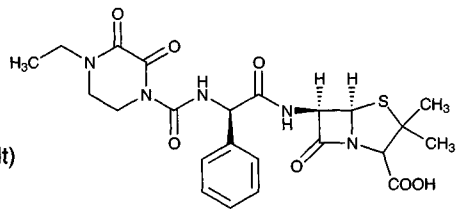
**OTHER SUBSTANCES**
**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine,

buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

## REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

# Piperacillin



**Molecular formula:** $C_{23}H_{27}N_5O_7S$
**Molecular weight:** 517.56
**CAS Registry No.:** 61477-96-1, 59703-84-3 (sodium salt)
**Merck Index:** 7616
**Lednicer No.:** 3 207; 4 179, 188

## SAMPLE

**Matrix:** aqueous humor
**Sample preparation:** 150 μL Aqueous humor + 30 μL 2.5 μg/mL cephalothin + 50 μL 400 mM HCl, mix, add 700 μL chloroform:1-pentanol 3:1, mix by swirl-mixing for 5 min, centrifuge at 300 g for 5 min, discard the organic layer. Centrifuge the aqueous layer briefly, hold it at 4°, inject a 20 μL aliquot.

## HPLC VARIABLES

**Column:** 250 × 4.5 5 μm Ultrasphere RP-ODS
**Mobile phase:** MeOH:water:buffer 40:48:12, the final pH was adjusted to 6.7 with triethylamine (Buffer was 50 mM pH 6.7 morpholinopropanesulfonic acid (MOPS)-triethylamine.)
**Column temperature:** 32
**Flow rate:** 1

**Injection volume:** 20
**Detector:** UV 254

### CHROMATOGRAM
**Retention time:** 6.86
**Internal standard:** cephalothin (4.74)
**Limit of detection:** 130 ng/mL

### OTHER SUBSTANCES
**Simultaneous:** acetaminophen, ampicillin, caffeine, salicylic acid, azlocillin, cefamandole, cefoxitin, cefuroxime, scopolamine, sulfamethoxazole, theophylline, ticarcillin, timolol
**Noninterfering:** acetazolamide, amitriptyline, atropine, carbachol, cefazolin, cefoperazone, cefotaxime, chlorpheniramine, codeine, diazepam, echothiophate, epinephryl borate, imipramine, prednisolone acetate, tropicamide, xylazine
**Interfering:** carbenicillin, mezlocillin

### KEY WORDS
rabbit

### REFERENCE
Riegel,M.A.; Ellis,P.P. High-performance liquid chromatographic assay for piperacillin in aqueous humor of the eye, *J.Chromatogr.*, **1988**, *424*, 177–181.

### SAMPLE
**Matrix:** bile, blood, urine
**Sample preparation:** Serum. 0.5 mL serum + 0.5 mL MeCN mix in 7 mL tube on vortex mixer; shake by rotation (20 rpm) 10 min; centrifuge 10 min 1000 g; transfer supernatant to another tube, add 7 aliquots dichloromethane; equilibrate 10 min; shake by rotation (20 rpm) 10 min; centrifuge 10 min 1000 g; inject aliquot of upper aqueous layer. Urine. Centrifuge urine and dilute 1:20. Bile. Centrifuge bile and dilute 1:10

### HPLC VARIABLES
**Column:** 150 × 4.6 5 μm Ultrasphere ODS
**Mobile phase:** 20:80 MeCN:20 mM ammonium acetate adjusted to pH 5 with glacial acetic acid
**Flow rate:** 1
**Injection volume:** 20
**Detector:** UV 214

### CHROMATOGRAM
**Retention time:** 5.6
**Limit of detection:** 500 ng/mL

### OTHER SUBSTANCES
**Also analyzed:** ampicillin, azlocillin, aztreonam, cefmenoxime, cefoperazone, cefsulodin, cefotaxime, ceftazidime, ceftriaxone, cloxacillin, desacetylcefotaxime, mezlocillin, penicillin G, ticarcillin

### KEY WORDS
serum

### REFERENCE
Jehl,F.; Birckel,P.; Monteil,H. Hospital routine analysis of penicillins, third-generation cephalosporins and aztreonam by conventional and high-speed high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *413*, 109–119.

### SAMPLE
**Matrix:** bile, blood, urine
**Sample preparation:** Plasma, serum. 200 μL Plasma or serum + 200 μL 25 μg/mL penicillin G in 50 mM pH 6.0 sodium phosphate buffer + 800 μL MeCN, vortex for 30 s, centrifuge at 3000 g for 10 min. Remove the clear supernatant and add it to 2 mL dichloromethane, mix for 30 s, centrifuge at 3000 g for 10 min, inject a 25 μL aliquot of the upper aqueous layer. Bile. 200

μL Bile + 400 μL 50 mM pH 7.0 sodium phosphate buffer + 2 mL MeCN, vortex for 30 s, centrifuge at 3000 g for 10 min. Remove the clear supernatant and add it to 2 mL dichloromethane, mix for 30 s, centrifuge at 3000 g for 10 min, inject a 25 μL aliquot of the upper aqueous layer. Urine. 100 μL Urine + 50 μL 5 mg/mL penicillin G in water, vortex for 30 s, make up to 10 mL with 50 mM pH 6.0 sodium phosphate buffer, inject a 25 μL aliquot.

**HPLC VARIABLES**
**Guard column:** 15 × 3.2 7 μm Brownlee C 18 guard column
**Column:** 250 × 4.6 5 μm Hypersil ODS (Keystone)
**Mobile phase:** Gradient. A was MeCN:10 mM sodium phosphate adjusted to pH 2.7 with concentrated phosphoric acid 3:97. B was MeCN:10 mM sodium phosphate adjusted to pH 2.7 with concentrated phosphoric acid 90:10. A:B from 95:5 to 50:50 over 9 min and then to 95:5 over 1 min.
**Flow rate:** 1.5
**Injection volume:** 25
**Detector:** UV 220

**CHROMATOGRAM**
**Retention time:** 11.8
**Internal standard:** penicillin G (12.5)
**Limit of quantitation:** 50000 ng/mL (urine), 1000 ng/mL (plasma, serum, bile)

**OTHER SUBSTANCES**
**Extracted:** tazobactam
**Simultaneous:** amoxicillin, ampicillin, cefoperazine, cefometazole, cefotaxime, cefotetan, cefuroxime, mezlocillin

**KEY WORDS**
plasma; serum

**REFERENCE**
Ocampo,A.P.; Hoyt,K.D.; Wadgaonkar,N.; Carver,A.H.; Puglisi,C.V. Determination of tazobactam and piperacillin in human plasma, serum, bile and urine by gradient elution reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *496*, 167–179.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 200 μL Serum + 50 μL 500 μg/mL mezlocillin + 200 μL 400 mM HCl + 3.5 mL dichloromethane, extract. Extract the organic phase with 200 μL 20 mM pH 6.2 phosphate buffer, inject a 30-60 μL aliquot.

**HPLC VARIABLES**
**Column:** C18
**Mobile phase:** MeCN:20 mM pH 3.0 sodium phosphate buffer 24:76
**Flow rate:** 1
**Injection volume:** 30-60
**Detector:** UV 254

**CHROMATOGRAM**
**Internal standard:** mezlocillin
**Limit of quantitation:** 250 ng/mL

**KEY WORDS**
serum; pharmacokinetics

**REFERENCE**
Harmsen,J.; Van Den Toorn,A. Determination of EDTA in water by high-performance liquid chromatography, *J.Chromatogr.*, **1982**, *249*, 379–384.

**SAMPLE**
**Matrix:** blood

**Sample preparation:** 500 μL Serum + methicillin + 2 mL MeCN, vortex for 1 min, centrifuge at 3000 g for 10 min. Remove the supernatant and add it to 5 mL dichloromethane, vortex, centrifuge at 3000 g for 10 min, inject a 15 μL aliquot of the aqueous phase.

**HPLC VARIABLES**
**Column:** 300 mm long μBondapak C18
**Mobile phase:** MeCN:100 mM pH 6.1 sodium phosphate buffer 25:75
**Flow rate:** 2.5
**Injection volume:** 15
**Detector:** UV 229

**CHROMATOGRAM**
**Internal standard:** methicillin
**Limit of detection:** 500 ng/mL

**OTHER SUBSTANCES**
**Extracted:** mezlocillin

**KEY WORDS**
serum; pharmacokinetics

**REFERENCE**
Martens,M.G.; Faro,S.; Feldman,S.; Cotton,D.B.; Dorman,K.; Riddle,G.D. Pharmacokinetics of the acyclureidopenicillins piperacillin and mezlocillin in the postpartum patient, *Antimicrob.Agents Chemother.*, **1987**, *31*, 2015–2017.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** Condition a 55 × 5 100-200 mesh AG 50W-X8 ($H^+$) column (Bio-Rad) with 10 mL MeCN:water 50:50. 600 μL Serum + 600 μL MeCN, vortex for 1 min, centrifuge at 2000 g for 5 min, add a 1 mL aliquot of the supernatant to the column, discard the first 200 μL effluent, collect the rest of the effluent. Remove a 450 μL aliquot and add it to 50 μL 10% sodium carbonate solution, heat at 60° for 1 h (to hydrolyse the β-lactam ring), cool in an ice bath. Remove a 100 μL aliquot and add it to 15 μL 200 mM pH 6.0 phosphate buffer, add 35 μL 80 mM 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole in MeCN, heat at 60° for 10 min, cool in an ice bath, add 30 μL 1 M HCl, inject a 5-10 μL aliquot.

**HPLC VARIABLES**
**Column:** 150 × 4.6 ODS-80TM (Tosoh)
**Mobile phase:** MeOH:100 mM pH 3.0 phosphate buffer 40:60
**Flow rate:** 1
**Injection volume:** 5-10
**Detector:** F ex 470 em 530

**CHROMATOGRAM**
**Retention time:** 7
**Limit of detection:** 50 ng/mL

**OTHER SUBSTANCES**
**Extracted:** methicillin, penicillin G
**Interfering:** penicilloic acid from piperacillin

**KEY WORDS**
derivatization; serum; SPE

**REFERENCE**
Iwaki,K.; Okumura,N.; Yamazaki,M.; Nimura,N.; Kinoshita,T. Precolumn derivatization technique for high-performance liquid chromatographic determination of penicillins with fluorescence detection, *J.Chromatogr.*, **1990**, *504*, 359–367.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 200 μL Serum + 50 μL 500 μg/mL mezlocillin + 200 μL 400 mM HCl + 3.5 mL dichloromethane, extract. Extract the organic phase with 200 μL 20 mM pH 6.2 phosphate buffer, inject a 30-60 μL aliquot.

**HPLC VARIABLES**
**Column:** C18
**Mobile phase:** MeCN:20 mM pH 3.0 sodium phosphate buffer 24:76
**Flow rate:** 1
**Injection volume:** 30-60
**Detector:** UV 254

**CHROMATOGRAM**
**Internal standard:** mezlocillin
**Limit of quantitation:** 250 ng/mL

**KEY WORDS**
serum; pharmacokinetics

**REFERENCE**
Klepser,M.E.; Patel,K.B.; Nicolau,D.P.; Quintiliani,R.; Nightingale,C.H. Comparison of the bactericidal activities of ofloxacin and ciprofloxacin alone and in combination with ceftazidime and piperacillin against clinical strains of *Pseudomonas aeruginosa*, *Antimicrob.Agents Chemother.*, **1995**, *39*, 2503–2510.

**SAMPLE**
**Matrix:** blood, dialysate
**Sample preparation:** Plasma. Mix 100 μL plasma with 200 μL MeOH containing 15 μg/mL IS, vortex for 15 s, centrifuge at 3000 rpm for 15 min, inject a 100 μL aliquot of the supernatant. Dialysate. Directly inject a 20 μL sample.

**HPLC VARIABLES**
**Guard column:** ODS
**Column:** 150 × 4.6 5 μm Regis C18
**Mobile phase:** MeCN:50 mM phosphate buffer 20:80, adjusted to pH 7.0
**Flow rate:** 1
**Injection volume:** 100
**Detector:** UV 220

**CHROMATOGRAM**
**Internal standard:** p-aminobenzoic acid propyl ester
**Limit of quantitation:** 2 μg/mL

**KEY WORDS**
plasma; rat

**REFERENCE**
Nolting,A.; Dalla Costa,T.; Vistelle,R.; Rand,K.H.; Derendorf,H. Determination of free extracellular concentrations of piperacillin by microdialysis, *J.Pharm.Sci.*, **1996**, *85*, 369–372.

**SAMPLE**
**Matrix:** blood, tissue
**Sample preparation:** Homogenize tissue in water at 4° for 30 s, centrifuge at 15000 rpm (Sorvall centrifuge) for 10 min. 100 μL Plasma or tissue homogenate + 100 μL 100 mM pH 5.5 $KH_2PO_4$ + 400 μL 25 μg/mL mezlocillin in MeCN, mix, centrifuge at 15000 rpm (Sorvall centrifuge), extract with 1 mL dichloromethane, inject 40 μL of the aqueous phase.

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm LiChrospher-C18
**Mobile phase:** MeCN:$NaH_2PO_4$ 20:80, pH 5.5

**Column temperature:** 37.5
**Flow rate:** 1.7
**Injection volume:** 40
**Detector:** UV 220

**CHROMATOGRAM**
**Retention time:** 5.5
**Internal standard:** mezlocillin (7.7)
**Limit of detection:** 386 ng/mL

**KEY WORDS**
plasma; fatty tissue; muscle; skin; appendix; intestinal mucosa; pharmacokinetics

**REFERENCE**
Kinzig,M.; Sörgel,F.; Brismar,B.; Nord,C.E. Pharmacokinetics and tissue penetration of tazobactam and piperacillin in patients undergoing colorectal surgery, *Antimicrob.Agents Chemother.*, **1992**, *36*, 1997–2004.

**SAMPLE**
**Matrix:** blood, tissue
**Sample preparation:** Plasma. 150 μL Plasma + 100 μL 400 μg/mL cloxacillin in water, vortex for 30 s, add 100 μL 10% trichloroacetic acid in water, vortex for 30 s, centrifuge at 3500 rpm for 5 min, inject a 200 μL aliquot of the supernatant. Tissue. Homogenize tissue in pH 7.4 Sörensen's buffer. 1 mL Tissue homogenate + 120 μL trichloroacetic acid, vortex for 30 s, centrifuge at 3500 rpm for 5 min. Remove the supernatant and add it to 5 mL chloroform, vortex for 1 min, centrifuge at 3500 rpm for 10 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 37°, reconstitute the residue in 250 μL mobile phase, inject an aliquot.

**HPLC VARIABLES**
**Column:** 150 × 4 5 μm reverse-phase RP-18
**Mobile phase:** MeCN:100 mM pH 6 potassium phosphate buffer 20:80
**Flow rate:** 2
**Injection volume:** 200
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** 3.20
**Internal standard:** cloxacillin (10.27)
**Limit of detection:** 500 ng/mL

**KEY WORDS**
plasma; brain

**REFERENCE**
Zarzuelo,A.; López,F.G.; Santos,M.; Lanao,J.M. Determination of piperacillin in biological samples by HPLC, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 601–610.

**SAMPLE**
**Matrix:** blood, tissue, urine
**Sample preparation:** Serum, plasma. Dilute serum or plasma 1:2 to 1:10 with buffer, centrifuge, inject a 20 μL aliquot of supernatant. Urine. Dilute urine 1:10 to 1:100 with buffer, centrifuge, inject a 20 μL aliquot of supernatant. Tissue (lung, gut). Cut tissue with a scalpel, homogenize with 1-3 mL buffer, centrifuge at 9600 g for 5 min three times, inject a 20 μL aliquot. Tissue (chondral). Cut tissue with a scalpel, homogenize with 3-6 mL buffer in an ice bath for 2-3 min, centrifuge at 9600 g for 5 min four or five times, inject a 100 μL aliquot. Dilute human pleural samples with buffer, centrifuge, inject a 20 μL aliquot. (Buffer was 66.6 mM $K_2HPO_4$ adjusted to pH 7.40 with $KH_2PO_4$.)

**HPLC VARIABLES**
**Column:** 200 × 4 5 μm Nucleosil C18

**Mobile phase:** MeCN:buffer 23:77, adjusted to pH 5.2 with phosphoric acid (Buffer was 57.4 mM $K_2HPO_4$ adjusted to pH 5.2 with phosphoric acid.)
**Flow rate:** 1
**Injection volume:** 20-100
**Detector:** UV 220

**CHROMATOGRAM**
**Retention time:** 11
**Limit of detection:** 100 ng/mL

**KEY WORDS**
serum; plasma; lung; gut; pleural; chondral

**REFERENCE**
Knöller,J.; König,W.; Schönfeld,W.; Bremm,K.D.; Köller,M. Application of high-performance liquid chromatography of some antibiotics in clinical microbiology, *J.Chromatogr.*, **1988**, *427*, 257–267.

**SAMPLE**
**Matrix:** blood, urine
**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

**HPLC VARIABLES**
**Guard column:** 20 mm long Symmetry C18
**Column:** 250 × 4.6 5 μm Symmetry C8 (Waters)
**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
**Column temperature:** 30
**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
**Injection volume:** 10-30
**Detector:** UV 200.5

**CHROMATOGRAM**
**Retention time:** 12.758

**KEY WORDS**
whole blood

**REFERENCE**
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

**SAMPLE**
**Matrix:** formulations
**Sample preparation:** 100 μL Solution + 4.9 mL MeOH:water 20:80, inject a 50 μL aliquot.

**HPLC VARIABLES**
**Guard column:** 5 μm Adsorbosphere C18
**Column:** 250 × 4.6 5 μm Adsorbosphere C18
**Mobile phase:** MeOH:100 mM ammonium acetate 43:57
**Flow rate:** 1
**Injection volume:** 50

**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** 12.0

**KEY WORDS**
stability-indicating; injections; 5% dextrose

**REFERENCE**
Inagaki,K.; Gill,M.A.; Okamoto,M.P.; Takagi,J. Stability of ranitidine hydrochloride with aztreonam, ceftazidime, or piperacillin sodium during simulated Y-site administration, *Am.J.Hosp.Pharm.*, **1992**, *49*, 2769–2772.

**SAMPLE**
**Matrix:** formulations
**Sample preparation:** Dilute 100:1 with saline, inject a 20 μL aliquot.

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm Adsorbosphere
**Mobile phase:** MeCN:10 mM $NaH_2PO_4$ 40:60, pH adjusted to 3.3 with 85% phosphoric acid
**Flow rate:** 1
**Injection volume:** 20
**Detector:** UV 220

**CHROMATOGRAM**
**Retention time:** 6.3

**KEY WORDS**
injections; saline; stability-indicating

**REFERENCE**
Choi,J.-S.; Burm,J.-P.; Jhee,S.S.; Chin,A.; Ulrich,R.W.; Gill,M.A. Stability of piperacillin sodium-tazobactam sodium and ranitidine hydrochloride in 0.9% sodium chloride injection during simulated Y-site administration, *Am.J.Hosp.Pharm.*, **1994**, *51*, 2273–2276.

**SAMPLE**
**Matrix:** formulations
**Sample preparation:** Dissolve in mobile phase so that the concentration of piperacillin is 2 mg/mL, add 25 μg methyl benzoate, inject a 10 μL aliquot.

**HPLC VARIABLES**
**Column:** 150 × 4.6 Ultron ODS-X
**Mobile phase:** MeCN:MeOH:10 mM tetrabutylammonium hydroxide and 5 mM potassium sulfate adjusted to pH 4.1 with phosphoric acid 300:25:1000
**Flow rate:** 0.7
**Injection volume:** 10
**Detector:** UV 270

**CHROMATOGRAM**
**Retention time:** 25.1
**Internal standard:** methyl benzoate (37.2)

**OTHER SUBSTANCES**
**Extracted:** YTR-830H, degradation products

**REFERENCE**
Tsukamoto,T.; Ushio,T. Determination of (2S,3S,5R)-3-methyl-7-oxo-3-(1H-1,2,3-triazol-1-ylmethyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4, 4-dioxide (YTR-830H) and piperacillin in pharmaceutical preparations by high-performance liquid chromatography, *J.Chromatogr.A*, **1994**, *678*, 69–76.

**SAMPLE**
**Matrix:** formulations
**Sample preparation:** Dilute 100-fold with saline, filter (0.2 μm), inject a 20 μL aliquot of the filtrate.

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm Adsorbosphere C18
**Mobile phase:** MeCN:100 mM sodium phosphate buffer 30:70 adjusted to pH 3.69 with 85% phosphoric acid
**Flow rate:** 1.2
**Injection volume:** 20
**Detector:** UV 220

**CHROMATOGRAM**
**Retention time:** 8-9

**KEY WORDS**
saline; injections; stability-indicating

**REFERENCE**
Chung,K.C.; Moon,Y.S.K.; Chin,A.; Ulrich,R.W.; Gill,M.A. Compatibility of ondansetron hydrochloride and piperacillin sodium tazobactam sodium during simulated Y-site administration, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 1554–1556.

**SAMPLE**
**Matrix:** formulations
**Sample preparation:** Dilute a 25-100 μL sample with 10 mL saline, filter (0.2 μm), inject a 20 μL aliquot.

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm Adsorbosphere C18
**Mobile phase:** MeCN:10 mM sodium phosphate 30:70 adjusted to pH 3.69 with 85% phosphoric acid
**Flow rate:** 1.2
**Injection volume:** 20
**Detector:** UV 220

**CHROMATOGRAM**
**Retention time:** 7.34

**OTHER SUBSTANCES**
**Simultaneous:** degradation products

**KEY WORDS**
injections; saline; 5% dextrose

**REFERENCE**
Moon,Y.S.K.; Chung,K.C.; Chin,A.; Gill,M.A. Stability of piperacillin sodium-tazobactam sodium in polypropylene syringes and polyvinyl chloride minibags, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 999–1001.

**SAMPLE**
**Matrix:** formulations
**Sample preparation:** Filter (0.2 μm) and inject an aliquot of the filtrate.

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm Adsorbosphere C18
**Mobile phase:** MeCN:10 mM sodium phosphate 40:60, pH adjusted to 3.3 with 85% phosphoric acid
**Flow rate:** 1
**Detector:** UV 220

**CHROMATOGRAM**
**Retention time:** 6.3

**OTHER SUBSTANCES**
**Simultaneous:** tazobactam

**KEY WORDS**
stability-indicating; 5% dextrose; injections

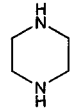
**REFERENCE**
Park,T.W.; Le-Bui,L.P.K.; Chung,K.C.; Rho,J.P.; Gill,M.A. Stability of piperacillin sodium-tazobactam sodium in peritoneal dialysis solutions, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 2022–2024.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

**HPLC VARIABLES**
**Guard column:** C18/Corasil (Waters)
**Column:** 300 × 3.9 μBondapak C18
**Mobile phase:** MeCN:10 mM ammonium acetate + 10 mM tetrabutylammonium bromide + 1% acetic acid 35:65
**Flow rate:** 1.5
**Injection volume:** 10-20
**Detector:** UV 230

**REFERENCE**
Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

# Piperazine



**Molecular formula:** $C_4H_{10}N_2$
**Molecular weight:** 86.14
**CAS Registry No.:** 110-85-0, 18534-18-4 (phosphate monohydrate), 14538-56-8 (phosphate), 142-88-1 (adipate), 144-29-6 (citrate), 41372-10-5 (citrate hydrate), 12002-30-1 (edetate calcium), 50322-15-1 (edetate calcium dihydrate), 133-36-8 (tartrate)
**Merck Index:** 7617

**SAMPLE**
**Matrix:** formulations
**Sample preparation:** Tablets. Grind tablets to a fine powder, weigh out amount equivalent to about 200 mg piperazine citrate, dissolve in 50 mL water, sonicate for 15 min, make up to 100 mL with water, filter (0.45 μm). Remove a 3 mL aliquot and add it to 2 mL 4 mg/mL 1-benzylpiperazine in acetone:water 50:50, add 10 mL of a filtered 5 mg/mL solution of dansyl chloride in acetone, add 10 mL base solution, mix, sonicate for 10 min, let stand in the dark for 30 min, add 20 mL water, add 20 mL chloroform, shake vigorously for 1 min, filter the chloroform layer through anhydrous sodium sulfate, dilute 2 mL of the filtrate to 5 mL with mobile phase, inject an aliquot. Syrup. Dilute 5 mL syrup to 100 mL with water, mix, measure out an aliquot equivalent to about 200 mg piperazine citrate, make up to 100 mL with water, mix. Remove a 3 mL aliquot and add it to 2 mL 4 mg/mL 1-benzylpiperazine in acetone:water 50:50, add 10 mL of a filtered 5 mg/mL solution of dansyl chloride in acetone, add 10 mL base solution, mix, sonicate for 10 min, let stand in the dark for 30 min, add 20 mL water, add 20 mL chloroform, shake vigorously for 1 min, filter the chloroform layer through anhydrous sodium sulfate, dilute 2 mL of the filtrate to 5 mL with mobile phase, inject an aliquot. Granules, powders (effervescent). Weigh out amount equivalent to about 200 mg piperazine citrate, slowly